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This report summarizes achievements gained during my visit in USA between 12 March and 20 May 1992. The main purpose was to exchange information in a field of bioremediation and industrial wastes biodegradation. To fulfill this objective, I presented a seminar about bioremediation in Czechoslovakia and worked in AFCESA laboratory on characterization of strain Pseudomonas fluorescens which was used in bioremediation projects in CSFR. In addition, I was allowed to visit the following laboratories and bioremediation sites: Gulf Breeze EPA laboratory, Hill AFB and an AFB in Alaska. Subsequent visit at University of Idaho (Department of Bacteriology, R. Crawford laboratory) was partly allowed so experiences there are also included in this report. This report is divided into two parts. First, one summarized laboratory methods of screening and testing of microorganisms and results of work on testing of the Pseudomonas fluorescens strain CCM 4427. Second one is a brief review of knowledge about bioventing.

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REPORT

Visit SPC 92-4008

David Klouda

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July 1992

Report: Visit SPC 92-4008

This report summarizes achievements gained during my visit in U.S.A. between March 12 and May 20 1992. The stay was allowed by kind invitation of Jim Spain, Ph.D., and Lt Col Neil Lamb and broad-minded grand extended by USAF.

The main purpose was to exchange information in a field of bioremediation and industrial wastes biodegradation. To fill this object I presented seminar about bioremediation in Czechoslovakia and worked in AFCESA laboratory on characterization of strain Pseudomonas fluorescens which was used in bioremediation projects in CSFR. In addition, I was allowed to visit following laboratories and bioremediation sites: Gulf Breeze EPA laboratory, Hill AFB and AFB in Alaska. Subsequent visit at University of Idaho (Department of Bacteriology, R. Crawford laboratory) was partly allowed by the grand so experiences from the work there are also incorporated in this report.

I gratefully acknowledge the kind hospitality and the expert expositions of Ms. Alison Thomas and Capt. Catherine Vogel that were my guides around the places mentioned above. Jim Spain and scientists from AFCESA laboratory helped me patiently with everything what I needed.

I will keep in touch with staff from Tyndall AFB. We will inform each other about interesting remediation projects and new technologies in our countries. I would be pleased to arrange meetings with Czechoslovak experts if somebody from Tyndall AFB come to Czechoslovakia.

The report is divided into two parts. First one summarized laboratory methods of screening and testing of microorganisms and results of work on testing of the Pseudomonas fluorescens strain CCM 4227. Second one is brief review of knowledge about bioventing. The report is written in general way - my personal experiences are presented together with facts reported in professional literature - in order to provide consistent overview of the problem.

Degradation of Aromatic Compounds by *Pseudomonas fluorescens* Strain CCM 4227

Introduction: Laboratory Methods Applied to Screening and Testing of Microorganisms Capable to Degrade Aromatic Compounds

Abounding source of microorganisms with interesting abilities are activated sludge from waste water treatment facilities and soil or water with long-term contamination. To increase in number microorganisms with desired capabilities sample is cultivated in liquid soil amended by given hazardous compound in fermentor operated in chemostat or batch mode. Batch cultivation can be applied to multiply suitable microorganism and then chemostat can be inoculated by resulting culture in order to select or induce the2 most effective strain. Increasing of hazardous compound concentration in chemostat input should be slow to allow microorganisms stepwise adaptation and avoid elution. Output analyses and microscopical control during process are necessary.

Substrate range of pure microbial culture can be tested on agar plates with mineral medium. Tested component if soluble can be added directly into the soil or dropped on the agar surface (auxonography). Microorganisms can also be supplied with carbon source in a vapor phase during cultivation of Petri dishes in desiccator or with small tube containing volatile substrate placed on the lid of inverted plate. In liquid cultures the supply of hazardous compound is provided by evaporation from tube attached in the head space of the flask or by aeration with compound vapor. The optimal way of testing is specific for each microorganism and hazardous compound. To confirm that given microorganism is able to utilize given compound as a sole carbon source, parallel negative (cultivation without the carbon source) and positive control (cultivation of a known strain that is able to utilize the compound under the same conditions) is useful to carry out (1).

The capabilities of *Pseudomonas fluorescens* strain CCM 4227 to degrade aromatic compounds were tested in this study. The strain when applied to bioremediation of soil and water contaminated by petroleum or petroleum products was able to accelerate substantially rate of decontamination.

Results and Discussion

Methods and results of P.fluorescens substrate range investigation are summarized in the table 1.

It can be seen that the range of aromatic compounds that P. fluorescens can utilize as a sole carbon and energy source is surprisingly small. The fact that P. fluorescens was capable of growth on catechols and not on any other aromatic compounds suggest that the microorganism does not possess enzymes responsible for conversion of benzene rings into dihydrodiols (dioxygenase) and catechols (dehydrogenase).

This conclusion seems to be at variance with experiments made 2 years ago: the strain

Compound	Method	Result
benzene	tube in Petri dish desiccator tube in flask	neg neg neg
toluene	desiccator	neg
phenol	0.05% in agar soil	neg
o-cresol	0.05% in agar soil	neg
m-cresol	0.05% in agar soil	neg
p-cresol	0.05% in agar soil auxonography	pos pos
catechol	auxonography	pos
4-methylcatechol	auxonography tube in Petri dish	neg pos
3-methylcatechol	auxonography tube in Petri dish	neg neg
benzoate	0.1% in agar soil	neg
ethylbenzene	tube in Petri dish	neg

Tab. 1.: Testing of Pseudomonas fluorescens. Cultures were grown at 28 °C in minimal salts medium (MSB) (2). Parallel positive control with Pseudomonas *sp. strain JS150 (3).*

was capable to degrade 80% of crude oil in liquid mineral medium in 21 days (from 30 $g_{hydrocarbons}/l$ to 5.63 $g_{hydrocarbons}/l$ - IR spektrophotometry). The inconsistency could be explained by degeneration of genetic material - the strain was stored on organic agar mediums for the last two years. Less likely, catabolism is able to degrade wider range of aromatic compounds in complex contaminants like crude oil by co-metabolism. The problem will perhaps be cleared up after repetition of the both experiments (substrate range investigation, degradation of crude oil) with original strain that was maintained in liquid nitrogen.

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Bioventing

Introduction

Industrial pollution of environment by organic matter has become world-wide problem. It is estimated that annual global input of petroleum is between 1.7 and 8.8 million tons (1) and value of released man-made chemicals (petroleum products, halogenated hydrocarbons) cannot even be approximated (2). Decomposition by microorganisms has been found to be one of the most effective tools for environment cleaning.
Biodegradation of hydrocarbons has been studied for more than 50 years (3). Scientists have found out huge degradation potential of naturally occurred microorganisms. Spectrum of biodegradable compounds is extended from single hydrocarbons chains (methane, ethane, propane...) to complicated aromatic and polyaromatic substituted compounds and is still being expanded (4). Besides naturally microorganisms special strains with exceptional abilities are constructed by genetic engineering tools. Safety roles cover the disposal of such strains so that they cannot usually be released into the environment. The microbial abilities has been maturely applied for cleaning of water and soil contaminated by organic compounds since 1970's (5). Conditions which facilitated fast bioremediation (nutrients and moisture addition, aeration, temperature) were created in excavated contaminated soils. A seed by suitable microbial culture slightly accelerated bioremediation in some cases (6). Increased costs, increased restrictions on land disposal and the need to remediate soil and ground water in depth and below surface structures made much more attractive *in situ* methods of decontamination (7). Depended on site conditions moisture, nutrients, microbial culture, surface-active compounds and electron acceptor are introduced into the subsurface to create optimal environment for microbial degradative action. Indigenous soil microorganisms have usually sufficient potential for degradation of the most common contaminants as petroleum and petroleum products. Nutrients addition also has small or no effect on *in situ* biodegradation in soil subsurface because the main limitation factor is an oxygen supply. In conventional methods water is used to carry oxygen into the subsurface. This approach has several disadvantages: large volume of water is needed (approximately 400,000 g of water per 1 g of hydrocarbons; high viscosity of water retards to attack soil micropores where the most contamination is occluded. These difficulties can be avoided neither by application of hydrogen peroxide solution nor other electron acceptor. The simplest solution of the problem is a use of air as the oxygen carrier. Systems for induction of air flow throw the contaminated underground soil were already well described as a basic attribute of another method of subsurface soil decontamination - soil vapor extraction.

Soil vapor extraction

Soil vapor or vacuum extraction (SVE) is method for removal of volatile compounds from soil subsurface by initiation of air flow through contaminated zone. General configuration of soil venting system is shown in Fig. 1. (8). There are three main factors governing the behavior of any *in situ* SVE system operation: vapor flow rate, contaminant(s) vapor concentration and vapor flow path relative to the contaminant

location. These factors can be predicted when the site characteristic (soil type and permeability, soil stratigraphy...) and contaminant characteristics (composition, boiling point distribution...) are known.

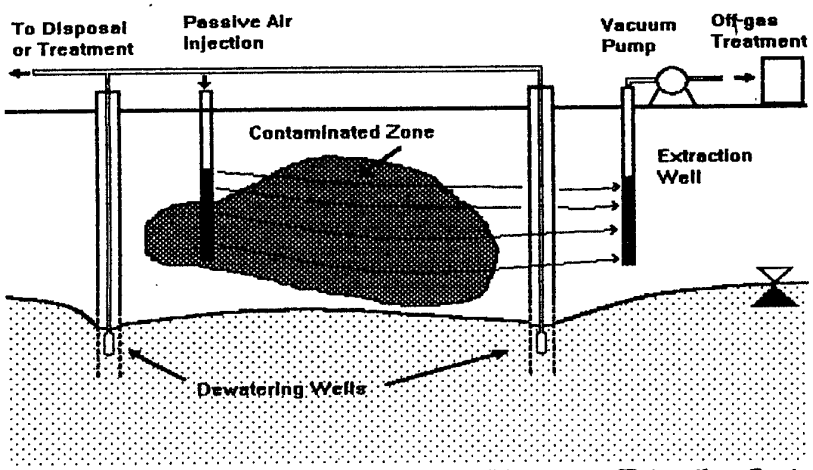

Figure 1.: General Configuration of Soil Vacuum Extraction System

Besides number, construction and location of extraction and injection wells, there are many other modifications available which can be considered during SVE-system design process, depending on site conditions. In order to focus on contaminated zone more precisely and increase extraction well radius of influence a discrete level vapor extraction device is used (9). Surface seal (polyethylene) is applied when the air suction from the surface should be avoided (10). Low soil permeability often retards the use of venting. Schuring *at al.* (11) described and patented a method and apparatus for pneumatic fracturing. The application of the device in soil with high silt and clay content increased soil permeability from 6 to 100 times (12). A special modification of SVE system configuration was described by Kaback *et al.* (13): active injection and extraction horizontal wells were used for remediation of saturated zone. The method for *in situ* stripping for simultaneous removal of contaminants from ground water and vadose zone by special wells is applied in Germany (14).
Off-gas treatment is selected according to composition and concentration of contaminants. Combustion (incineration) is economical for the highest concentrations - about 10,000 ppm. Catalytic oxidation of heated vapor can be used for vapor concentrations less then 8000 ppm. Carbon beds are the most common mean of vapor treatment. They become economical for low concentration - about 500 ppm (7). Costs of off-gas treatment constitute substantial part of total SVE expenses, in most cases at least 50 percent (15).

Bioventing system

The method of bioventing applies SVE well system for supply oxygen to soil microorganisms in contaminated zone in order to decompose contaminants by biodegradation. The system can further include device for nutrients and/or moisture addition. An infiltration through injection wells (16) or network of perforated tubes located close to ground surface above contaminated zone (17) is usually applied.

Similar device is used for soil temperature regulation by distribution of warm water at Air Force Base in Alaska (18). S. Coffa *et al.* (16) injected heated air (35-40 °C) in order to speed up bioremediation. Surface insulation can slightly slow down surface insulation during winter (18).
The effect of moisture and nutrients addition is questionable. Moisture can facilitate microbial action (19) but higher water content (above 50% of soil capacity) bar oxygen supply. Nutrient addition increased microbial activity in laboratory tests (19) but effect was negligible in field applications (17). Recycling of N-, P- and other sources is expected during relative slow bioventing process (20). Temperature substantially influences rate of bioventing process. R. N. Miller *et al.* (17) observed correlation between biodegradation rate and temperature corresponding to Hoff-Arrhenius equation.

Monitoring and Operation of Bioventing System

Several parameters have to be monitored during bioventing process in order ensure its efficient operation.
Vapor flow rate from extraction wells and/or into injection wells.
Contaminant concentration and composition in off-gas. Total volatilization and cumulative amount of contaminant, removed by volatilization can be calculated. In addition, contaminant concentration must be controlled to not excess safety level and level given by regulatory. Changes of off-gas composition in the case of complex contaminants like petroleum, gasoline and oils can illustrate progress of decontamination.
Carbon-dioxide production and/or oxygen consumption is estimated by comparison CO_2 and/or O_2 input concentrations (ambient air) and concentration in the off-gas. Decomposition rate and cumulative amount removed by microbial action can be calculated. Better estimate of contaminants biodegradation rate can be made when naturally occurred respiration is taken into account. It means to measure also CO_2 and/or O_2 concentrations in uncontaminated site under the same conditions. R.E.Hinchee and R.N. Miller, 1991 (21), found out O_2 consumption measurement method more consistent then CO_2 production measurement one.
Contaminant concentration and composition in soil-gas. At least three soil-gas monitoring points is recommended to build up: one at the origin edge of contaminated zone, one near the extraction well and one somewhere between them (7). Analyses of soil gas can provide valuable information about movement of contaminated zone and microbial activity.
Ambient and soil temperature.
Results of the measurements give almost complete picture of bioremediation process that is very important for determining when to turn off the system. Contaminant(s) concentration in soil gas is the most useful in the case of volatile compounds. Biodegradation of non-volatile parts of contamination (O_2 consumption and/or CO_2 production) must be taken into account in other cases. When all parameters indicate reaching of clean up target, we can curry out confirmation soil boring.

Field-scale investigation

Two large-scale bioremediation projects conducted by AFCESA which may suggest new trends in development of bioventing technology, are described in this chapter. Both studies are not closed and their results have not been presented till this time.

Hill Air Force Base

Very interesting field bioventing experiment is currently running at Hill AFB, UT. JP-4 contamination of soil occurred in the depth of about 20 m. Concentration was ranging between 8,000 and 10,000 ppm. There was no danger of fast movement of contaminated zone. Bioventing system was designed which consisted of only one injection well and two soil-gas monitoring points in distance of approximately 20 and 40 m from well. Flow rate of injected air is very low - 0.027 m^3/minute. Soil moisture and nutrient content was assumed to be sufficient (recycling of N- , P- and other mineral sources is expected during slow process). In addition any further fertilizers or moisture supply could hardly be done under the site condition. Biodegradation will be probably very slow. It is expected that complete decontamination takes three to four years. Low rate is compensated by extremely low costs. Preliminary results are expected to be reported later this year.

Air Force Base at Alaska

Field study at Alaska's AFB is conducted by AFCESA and EPA. The main objective is to investigate applicability of bioventing technology under cold climate conditions. Three bioventing plots were built up at the site where JP-4 spill occurred in the depth of 5 m. EPA plot consists of air injection well, three-level soil-gas probes, three-level thermocouples, surface insulation and active thermoregulation system, AFCESA plot is without the active thermoregulation and control plot has no thermoregulation. In active warming system of EPA plot water is pumped from extraction well to instantaneous water heaters. Warm water circulates through the loop of soaker hose and then back out of the sewer pipe through a return manifold and back to the well. Preliminary results (November 1992) indicated almost no temperature decrease in this plot during the winter (about 12 °C). Temperature in passive plot was slightly higher (2 to 4 °C) then in control one (0 to 2 °C). O_2 utilization, CO_2 production and biodegradation rates were calculated from CO_2 and O_2 concentration changes in soil gas samples during respiration tests. Biodegradation rates in active plot (about 15 mg/kg.day) were approximately ten times higher then in the other plots.

Conclusion

The bioventing is a cheap and effective in situ decontamination method for subsurface soil and ground water polluted by biodegradable organic compounds. It is more effective than traditional in situ bioremediation approaches because of higher oxygen concentration in and lower viscosity of air in comparison with water. It does not require off-gas treatment that makes SVE method at least two time more expensive.

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